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Comparative Study of Conventional Papanicolaou Smear and Cytospin Smear in Cervical Cancer Screening

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Abstract

Context: Cervical carcinoma is the fourth common malignancy in women worldwide and second most common in developing countries including India. Cytological screening leads to a reduction in the rate of invasive cancer of uterine cervix. The present study highlights the importance of cytospin method of liquid based cytology which improves the efficacy of Papanicolaou smears. *Objectives:* To compare efficacy of cytospin Papanicolaou smears with conventional Papanicolaou Smears in cervical cancer screening based on Bethesda system of classification, 2014. *Materials and Methods:* A prospective study of cervical cytology of 134 samples was carried out on women from age group of 18-65 years. Using Ayre's spatula one slide was prepared and immediately fixed in fixative. The residual material was rinsed in fixative then spun in cytospin to obtain direct smear. Both conventional and cytospin smears were stained by Papanicolaou stain. *Results:* Of the 134 cervical cytospin smears studied, 87.9% (118 cases) were non-neoplastic, 9.6% (13 cases) were neoplastic lesions and 2.2% (3 cases) were unsatisfactory. The most common neoplastic lesion was LSIL and HSIL accounting for 3% (4 cases) each followed by ASCUS, squamous cell carcinoma and adenocarcinoma. Cytospin showed significant difference in the morphological features compared to conventional. *Conclusion:* Cytospin method of liquid based cytology is recommended as it improves the sample quality, morphology and also cost effective. It is an alternative screening method in low resource settings, like developing countries including India where women are at high risk for developing cervical cancer.

Keywords: Cervical Carcinoma; Conventional Pap Smear; Cytospin Pap Smear; Liquid Based Cytology.

Introduction

Cervical cancer is 2nd most common malignancy among women in India [1]. Cytological screening leads to reduction in the rate of cervical cancer. The sensitivity of the conventional Pap smears for the detection of cervical cancer is less due to several limitations. To overcome these, liquid-based cytology (LBC) came into existence [2].

ThinPrep® and SurePath® are the two techniques approved by Food and Drug Administration (FDA) [3]. But have high capital investments for instruments and high cost per test. Alternately LBC with cytospin has been tried [4].

The present study aimed at comparing efficacy of cytospin smear with conventional Papanicolaou smear in cervical cancer screening based on Bethesda system of classification, 2014.

Materials and Methods

In the present study, cervical smears were prepared using the cytospin method of liquid-based cervical cytology and compared with the conventional Pap smears. A prospective study of 134 samples was carried out in the Department of Pathology by split smear technique for conventional Pap smear and

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cytospin smears.

Cervical cytology samples from all women from 18-65 years attending the Obstetrics and Gynaecology (OBG) Out Patient Department with presenting complaints of white discharge per vagina, post-coital bleeding, mass per vagina, pain abdomen, irregular menstruation, infertility and for routine cervical cancer screening in high risk patients like coitus before the age of 18yrs, multiple sexual partners, delivery of 1st baby before the age of 20years, multiparity with poor birth spacing between pregnancies, poor socioeconomic status, women with STD, HIV infection, herpes simplex virus 2 infection, human Papilloma virus infection [16,18,31,33] or condylomata were included in the study and detailed examination was done.

Non co-operative patients or patients who did not give consent and technically defective slides like broken slides and slides having drying artefacts were excluded from the study.

In all these cases after a detailed history and thorough clinical examination, conventional Pap smears were prepared from cervix with an Ayre's spatula and one slide was prepared and immediately fixed in 100% methanol. Then residual material on spatula were rinsed in 5ml of 100% methanol. It was allowed to sediment for 1 hour, supernatant was decanted and around 100 microlitre of the sediment material was transferred into cytofunnel with filter paper placed between slide and funnel and spun in Cytospin [MedSpin4] at 800rpm for 5 minute. Both conventional and Cytospin smears were stained by Papanicolaou stain.

The smears prepared by the conventional method and cytospin method were observed for the following parameters like cellularity, cellular overlapping, morphological changes, clear background, nuclear features and endocervical cells. The Bethesda system of classification, 2014 was used to report the smears.

All characteristics were summarized descriptively. For continuous variables, the summary statistics of normal (N), mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries. Chi-square (χ^2) / Fisher exact test was employed to determine the significance of differences between groups for categorical data. The difference of the means/proportion of analysis variables was tested with the t-test/z test and ANOVA. If the p-value was < 0.05, then the results were considered to be significant. Data were analyzed using SPSS software v.23.0.

Results

In the present study 134 cervical cytology smears were prepared using the cytospin method of liquid-based cervical cytology (LBC) and compared it with the conventional Pap smears and morphological features were observed.

Age group of patients ranged from 18 to 65 years with the youngest patient aged 18 years and the oldest 65 years with a mean age of 38.3 years. Majority of the patients were in the age group of 31-40 years. The most common clinical presentation was white discharge per vagina followed by irregular cycles and menorrhagia. Satisfactory cell samples were obtained in 94.8% (127cases) of conventional Pap smear and 97.8% (131 cases) of cytospin Pap smear.

In the present study 134 cervical smears prepared by conventional were studied, out of which 91.3% (116 cases) were reported as non-neoplastic lesions and 8.7% (11 cases) were reported as neoplastic lesions. Out of 134 cervical smears prepared by cytospin studied, 90.1% (118 cases) were reported as non-neoplastic lesions and 9.9% (13 cases) were reported as neoplastic lesions.

By conventional technique, the most common neoplastic lesion was found to be ASCUS accounting for 3% (4 cases), followed by LSIL 2.2% (3 cases), HSIL 2.2% (3 cases) and ASCUS-H) 0.7% (1 case). By the cytospin technique, the most common neoplastic lesion was found to be LSIL, HSIL accounting for 2.2% (4 cases) each, followed by ASCUS 2.2% (3 cases), squamous cell carcinoma 0.7 % (1case) and papillary adenocarcinoma 0.7% (1 case).

By conventional technique, the most common non-neoplastic lesion was inflammatory smear 48.5% (65 cases) followed by normal study 19.4% (26 cases), bacterial vaginosis 11.9% (16 cases), candidal infestation 3.7% (5 cases), trichomonas vaginalis 1.5% (2 cases), atrophic smear 1.5% (2 cases). By the cytospin technique, the most common non-neoplastic lesion was inflammatory smear 47% (63 cases) followed by normal study 20.1% (27 cases), bacterial vaginosis 12.7% (17 cases), candidal infestation 3.7% (5 cases), trichomonas vaginalis 2.2% (3 cases), atrophic smear 1.5% (2 cases), leptothrix infestation 0.7% (1 case). (Table 1).

In present the study, histopathology correlation was not done. Taking cytospin as standard, sensitivity and specificity of conventional Pap smear was calculated and found to be: Sensitivity-84.6%, Specificity-100%, Positive Predictive Value (PPV)-100%, Negative Predictive Value (NPV)- 98.4% and accuracy (ACC)-

98.5% [True positive (TP)=11, True negative (TN) = 121, False positive (FP) =2, False negative (FN) = 0]. Cellularity was adequate in most of the cytospin smears while the number of unsatisfactory smears were more in CPS. Cellular morphologic change was present in most of CPS samples. Cellular overlapping and inflammatory infiltrate were prominently present in CPS but decreased in cytospin smears. Clean background was observed in most cases of cytospin smears which was not seen in majority of CPS. Nuclear changes were very clear by cytospin smears, but not very clear by CPS. Endocervical cells were more in CPS than in cytospin [Table 2].

Table 1: Comparison of morphological distribution of total number of cases studied on conventional Pap smear and cytospin Pap smear

Morphological Distribution		Conventional Pap smear		Cytospin Pap smear		p value
		N	%	N	%	
Neoplastic	ASCUS	4	3.0	3	2.2	0.702
	LSIL	3	2.2	4	3.0	0.702
	HSIL	3	2.2	4	3.0	0.702
	ASCUS-H	1	0.7	0	0.0	0.316
	Squamous Cell carcinoma	0	0.0	1	0.7	0.316
	Papillary adenocarcinoma	0	0.0	1	0.7	0.316
Non Neoplastic	Inflammatory Smear	65	48.5	63	47.0	0.807
	Inflammatory Smear-Leptothrix	0	0.0	1	0.7	0.316
	Atrophic smear	2	1.5	2	1.5	--
	Trichomonas Vaginalis	2	1.5	3	2.2	0.652
	Candida	5	3.7	5	3.7	--
	Bacterial vaginosis	16	11.9	17	12.7	0.853
	Normal study	26	19.4	27	20.1	0.878
Unsatisfactory		7	5.2	3	2.2	0.197
Total		134	100.0	134	100.0	

Table 2: Comparison of morphological features in CPS and Cytospin Pap smear

Morphological features	Conventional Pap smear		Cytospin Pap smear		P value
	N	Percent (%)	N	Percent (%)	
Cellularity present	131	97.8	133	99.3	0.315
Absence of cellular overlapping	99	73.9	119	88.8	<0.001*
Absence of morphologic changes	123	91.8	131	97.8	<0.004*
Clean background	109	81.3	130	97	<0.001*
Better nuclear features	126	94	128	95.5	0.585
Presence of Endocervical cells	50	37.3	47	35.1	0.704
Total	134	100	134	100	

Note: *-Difference is statistically significant at 5% level of significance

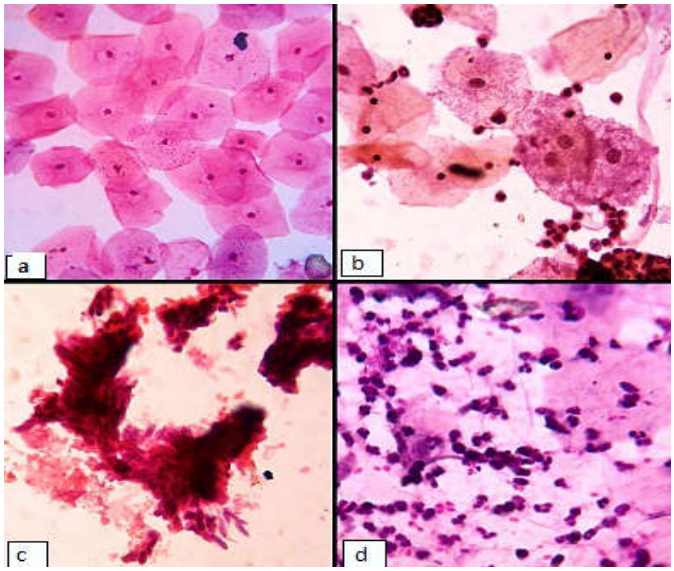

Fig. 1: Cytospin smears **a)** Normal study- Squamous cells showing clear nuclear features. **b)** Bacterial vaginosis-Clue cells in a clear background, **c)** Papillary Adenocarcinoma- Pleomorphic cells in clusters.CPS **d)** Candidiasis-Squamous cells showing overlapping with candida and obscuring inflammatory cells. (Pap400X)

Discussion

The development of conventional Papanicolaou smear (CPS) have lead to increased detection of pre-cancerous and cancerous lesions of the cervix but has limitations like unsatisfactory smears, drying artefacts and obscuring factors like inflammatory cells and hemmorhage [5]. To overcome these limitations, liquid-based cytology (LBC) came into existence [6].

Liquid-based preparations (LBP) were originally developed by the Germans in the early 70s to minimize cell overlap for better performance of automated screeners in the identification of abnormal cells. Eventually they have almost replaced the use of conventional cervicovaginal smears in the developed world after successful clinical trials. ThinPrep® and SurePath® are the two FDA approved liquid-based methods that are used for the preparation of such smears. Most developed countries have employed these systems for routine cervical screening. SurePath® works on the principle of density gradient centrifugation and ThinPrep® on membrane filtration. LBC is a technique achieved by rinsing the collection device in a preservative/fixative fluid to generate a suspension of cells that is processed to deposit a monolayer of cells on a microscope slide. Both these systems result in the formation of a small circular smear on the slide after the sample is placed in a fixative solution and processed by the machine. The advantages being: relative absence of blood and debris, monolayer formation, better quality of smears and smaller field for screening thus accelerating the screening process. The cells show a high contrast between the nucleus and cytoplasm with a clear background and thus requiring special training in interpretation of material [5,7].

The disadvantages of closed systems like SurePath® and ThinPrep® includes huge capital investment for equipment and logistics. The proprietary reagents adds on to the maintenance and increases per test kit cost further significantly. Cytospin method of manual liquid based cytology is a relatively inexpensive and cost-effective method of LBC comparable with conventional Pap smears (CPS). Hence we have adapted this technique to assess its adequacy and utility in routine screening.

In the present study, we have used the cytospin which is a manual method of Liquid-based Cytology. The cervical smears were taken using wooden Ayre's spatula. Using the split sample technique, the cells were smeared onto a glass slide for CPS and remaining cells were rinsed into a liquid fixative vial i.e,100% methanol. The sample was left for 1hour for sedimentation. The sample was centrifuged with cytospin to obtain a direct smear. The cells were seen uniformly dispersed onto the glass slide with a cell deposition area of 6X6 mm with increased cell yield [8]. The slide was fixed with 100% methanol and stained with the Papanicolaou stain. In this way, 134 cases were sampled and smears obtained were compared with the corresponding conventional Pap smears.

In the present study, the youngest patient was 18 and the oldest 65 years. The mean age of the patients was 38.3 years similar to studies by authors like Jena et al [9] and Verma K et al [10]. The most common presenting symptom of the patients in present study was white discharge per vagina which was similar to other studies by authors like Sherwani et al [2], Bukhari et al [11]. Other common symptoms were irregular cycles and menorrhagia. Specimen adequacy was significantly increased in present study as also observed by Lee KR et al [12], Ronco G et al [13] and Hutchinson et al [14].

Many authors have reported decreased rate of unsatisfactory smears by liquid based cytology owing to optimal fixation and easy monitoring of preparation quality. Also, another advantage is that the unsatisfactory samples can be reprocessed [3]. In the present study reprocessing was done in one case, adequate material was obtained and diagnosed as adenocarcinoma. Studies by Bishop JW et al [15], Fremont-Smith M et al [16], Marino JF et al [17] and Utagawa ML et al [18] have reported an decrease in the unsatisfactory rates while studies by Davey E et al [19] have found no reduction in proportion of unsatisfactory smears. Weynand et al [8], however found that endocervical cells were absent in 5.3% of the cases which was attributed to a bias in methodology.

In the present study, we found that 3 cases (2.2%) of cytospin were unsatisfactory compared to 7 cases (5.2%) on CPS. Hence there was a significant decrease in the number of unsatisfactory samples agreeing with studies by Sherwani RK et al [2], Rimiene J et al [3] and Nandini NM et al [20].

A study by Hutchinson et al [14], documented that the ThinPrep® method had heightened the sensitivity significantly when compared with CPS. They also reported a percentage agreement of 85.8% between ThinPrep® and conventional smear diagnosis.

The sensitivity of CPS as found by Hussein et al [21] was 82% versus 92% by LBC while the specificity was 76% versus 83% by LBC. Hence the sensitivity and specificity was better with LBC when compared to conventional smears. However, they recommended

larger studies to verify the findings. They observed 73% agreement between conventional and LBC preparations.

In the present study cytospin Pap smears showed high detection of carcinomas hence keeping that as standard sensitivity and specificity of CPS were calculated. The sensitivity and specificity of CPS was 84.6% and 100% respectively. The percentage agreement between cytospin Pap smear and conventional Pap smear was 94.03%.

In the present study, we found an increased detection rate for LSIL, HSIL, squamous cell carcinoma and adenocarcinoma with cytospin smears when compared to conventional smears. The overall sensitivity was improved owing to better sampling technique, absence of obscuring factors, decreased reading time , better preservation of cells and also prevents drying artefacts due to direct fixation of cells in the liquid medium. Similar increase in sensitivity was reported in two different studies by Sherwani et al [2]. Most studies including present showed an overall increased detection rate of epithelial cell abnormalities by the LBC method. The percentage agreement between LBC and conventional smears in present study was 94.03% which was similar to studies by Hussein et al [21]. Another advantage of cytospin Pap smear includes decreased reading time because of smaller dimension of 6X6 mm in the present study. Deshou H etal [22] prepared slide manually with dimension with a dimension of 15-17mm. Alves VAF etal [23] in their study on automated method of LBC showed the dimensions of smears being 20mm by ThinPrep®, 13mm by Autocyte® Prep and 25mm by DNACITOLIQ®.

In the present study, we found a good correlation between cytospin and CPS in the detection of infectious agents. A total of 26 cases (19.3%) of infectious diseases by cytospin smear comprising of 17 cases (12.7%) of bacterial vaginosis, 5 cases (3.7%) of candida, 3 cases (2.2%) of trichomonas and 1 case (0.7%) of leptothrix. CPS detected 23 cases (17.1%). One case of bacterial vaginosis, one case of trichomonas and one case leptothrix was missed on CPS. Similarly Sherwani RK et al [2] also reported enhancement of microscopic details of infectious agents like candida, coccobacilli and trichomonas.

In the present study, morphological features between CPS and cytospin Pap smears were compared. Cytospin showed better cellularity in 133 cases (99.3%), absence of cellular overlapping in 119 cases (88.8%), absence of morphological changes in 131 cases (97.8%), clean background in 130 cases (97%), better nuclear features in 128 cases (35.1%) compared to conventional Pap smears having 131 cases (97.8%), 99 cases (73.9%), 123 cases (91.8%), 109 cases (81.3%) and 126 cases (94%) respectively. Morphological features like absence of cellular overlapping , absence of morphological changes and clean background showed p value less than 0.005 which was statistically significant at 5% level of significance (Table 2). Similar study done by Siebers AG et al [24] showed significant difference between the morphological features in LBC, compared to conventional cervical cytology.

Various studies have reported a higher percentage of endocervical component in conventional cases which has been attributed to the split-sample collection protocols and use of the residual sample for the LBPs [25]. This can be prevented by direct-vial sampling which allows the entire cervical sample to be rinsed into the liquid-preservative fluid, allow an equal percentage of thin-layer slides to have the endocervical component when compared to CPS [6,16]. The technique used in the present study was split-sample technique and hence there was no increase in rates of endocervical component on cytospin smears. In contrast, endocervical cell component was decreased in the cytospin Pap smears in the present study. Similar limitations were seen in a study by Nandini NM et al [20].

The following are the advantages of liquid-based cytology- the provision for long term storage of the liquid sample, thin monolayer of cells within a clean background attributed to fixative used [7]. Thus liquid-based cytology improves the quality of screening of slides by giving a clear background and removal of obscuring factors and also by reducing reading time [25].

Besides, cytospin Pap smear method of MLBC has other advantages as the residual specimens can be used for ancillary testing like detection of HPV-DNA by PCR or In-situ DNA Hybridization and Immunocytochemistry by cell block preparations [26].

Numerous automated computer assisted systems have been developed for screening of slides for abnormal cells like the PAPNET system and Focal point analyser which reduces the screening time and increases the detection rate but is an expensive method to be employed for routine screening in a country like ours [27].

Limitations of the study were that split sample method for sample collection was used which could lead to ineffective sampling as we found endocervical component were less in cytospin smears when compared with CPS which can be attributed to the split sample collection protocol. This can be overcome by direct sampling method and sampling by

experienced gynecologists [16,20].

Another limitation of the study was that histopathological correlation. Sensitivity and specificity could have been better achieved when there is histopathological correlation.

Conclusion

Cervical cancer is the second most common malignancy among women worldwide and commonest in India. Cytological screening leads to a reduction in the rate of invasive cancer of uterine cervix. Conventional Pap smear screening for detection of cervical cancer is less sensitive due to several limitations. Cytospin method is an inexpensive, cost effective technique of liquid based cytology method compared to ThinPrep® and SurePath®. It is recommended, as it improves the sample quality, reduces the likelihood of false negative results and better morphology. It over comes the limitation of conventional smear as it significantly reduces unsatisfactory smears, improves specimen adequacy, detects more intraepithelial lesion. It is of value as an alternative, more effective screening strategy in low resource settings, like developing countries including India where women are at high risk for developing cervical cancer. Thus it will significantly improve early detection and treatment of cervical lesions. Also, further ancillary testing like HPV DNA by PCR or insitu DNA hybridization and immunocytochemistry can be performed with the remaining sample.

Key Messages

Cytological screening leads to a reduction in the rate of invasive cancer of uterine cervix. Conventional Pap smear screening for detection of cervical cancer is less sensitive due to several limitations. Cytospin method is an inexpensive, cost effective method of liquid based cytology compared to ThinPrep® and SurePath®.

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